(11) Japanese Patent Application Laid-Open No.60-47962

(43) Laid-Open Date: March 15, 1985

(21) Application No.58-154922

(22) Application Date: August 26, 1983

(72) Inventor: Yoshitaka ITO

(71) Applicant: TERUMO CORPORATION

(74) Agent: Patent Attorney, Kousuke NISHIMURA

(54) [Title of the Invention] Methods for measuring blood antigen or antibody levels and reagents employed for the same

SPECIFICATION

1. Title of the Invention

Methods for measuring blood antigen or antibody levels and reagents employed for the same

2. Claims

(1) A method for measuring the blood level of an antigen or antibody comprising adding a hemolytic agent and an antigen- or antibody sensitization carrier suspension to a whole blood sample and monitoring the aggregation reaction.

(2) A reagent used for measuring the blood level of an antigen or antibody comprising a hemolytic agent and an antigen- or antibody sensitization carrier.

(3) The reagent according to Claim 2 wherein the hemolytic agent is a saponin.

3. Detailed Description of the Invention

I. Background of the Invention

Field of the Invention

The present invention relates to a method for measuring the blood level of an antigen or antibody as well as a reagent employed for the same.

More particularly, the invention relates to a method for measuring the blood level of an antigen or antibody by means of an aggregation reaction based on an immune reaction

as well as a reagent employed for the same.

The invention is utilized in various immunological tests such as the diagnosis of rheumatoid arthritis.

Prior Art and its Problems

A conventional method for measuring an antigen or antibody in a blood on the basis of the aggregation reaction employs as a sample a serum because of the difficulty in a macroscopic assessment of the aggregation when the sample contains red blood cells. However, the preparation of the serum requires a complicated and time-consuming process for example when handling a large number of the samples. It is also required, in preparing the serum, to collect the blood in an amount exceeding the amount required actually in the test.

II. Objective of the Invention

An objective of the invention is to provide a method for measuring the level of an antigen or antibody directly using a while blood without any tiresome process for preparing a serum sample.

Another objective of the invention is to provide a reagent used in the method described above.

For the purpose of achieving such objectives, the invention consists of a method for measuring the blood level

of an antigen or antibody comprising adding a hemolytic agent and an antigen- or antibody sensitization carrier suspension to a whole blood sample and monitoring the aggregation reaction.

Furthermore, the invention consists of a reagent used in the method mentioned above comprising a hemolytic agent and an antigen- or antibody sensitization carrier.

Moreover, the invention consists of the reagent comprising as a hemolytic agent a saponin.

III. Detailed Description of the Invention

A method according to the invention can be conducted by adding a hemolytic agent and an antigen- or antibody sensitization carrier suspension to a sampled whole blood and monitoring the aggregation reaction.

The hemolytic agent employed in the method mentioned above may for example be a saponin or various surfactants. The hemolytic agent may be added to the whole blood prior to the aggregation reaction to hemolyze red blood cells, or may be added at a concentration of about 0.2 to 2% to the antigen- or antibody sensitization carrier suspension for effecting the hemolysis of the red blood cell upon the aggregation reaction. The antigen- or antibody sensitization carrier is not limited particularly and may be any known material such as a latex resin, inorganic

adsorbent, chemically treated immobilized erythrocyte and the like.

The monitoring of the aggregation reaction can be conducted by the law of the art. Thus, a drop of a whole blood was added dropwise onto a glass slide, to which each one drop of a hemolytic agent and an antigen- or antibody sensitization carrier suspension, mixed thoroughly using a wood rod, and spread over an area of about 20 x 25 mm. The glass slide was held by both hands, shaken gently for 1 minutes, and then examined for any aggregation visually. This visual evaluation is not affected by red blood cells, which have already been lyzed.

The invention is further described in the following Examples.

EXAMPLES

(1) Preparation of human gamma-globulin sensitization latex for detecting rheumatoid factors (RF)

A polystyrene latex (particle size: 0.117 „) is suspended in a glycine-sodium chloride buffer solution (pH 8.2) (hereinafter abbreviated as GNB) at the solid content of 2.0%. On the other hand, a human gamma-globulin which has been dialyzed against GNB was dissolved in GNB at the concentration of 10 mg/ml. The both solutions were mixed in the volume ratio of 1:1, and warmed at 50°C for 1 hour. The

resultant solution was washed by centrifugation (17,000 rpm, 10 minutes), combined with GNB containing 0.5% bovine serum albumin and 0.4% saponin to prepare a 0.4% sensitization latex suspension. Under the condition described above, the sensitization protein concentration was 10 to 100 „gN/ml and the latex particle density was 4.53×10^8 particles/ml, allowing for an assumption that approximately 75,000 molecules of gamma-globulin bind to a single latex particle.

(2) Slide aggregation reaction

A drop of the sensitization latex obtained in (1) described above (about 0.02 to 0.03 ml) and a drop of a blood or serum were mixed thoroughly on a glass slide for reaction, spread over an area of about 2 cm in diameter, and subjected to the aggregation reaction. The glass slide was swung back and forth, and examined after 1 minutes for any aggregation reaction as well as the degree, if any, based on the criteria shown below. The results are shown in Table 1.

Positive (+)

Aggregation clots are observed throughout the wet region, with the aggregation being marked macroscopically.

Negative (-)

No aggregation was observed macroscopically.

Judgement impossible (?)

The latex aggregation is unclear.

Positive control

Rheumatoid arthritis (RA) control serum (aggregation titre: 160)

Negative control

Normal healthy human serum

The control serum described above and normal healthy human concentrated red blood cells were reconstituted (hematocrit: 40%) and used as a sample.

Table 1

	Sensitization latex sample	Saponin-supplemented sensitization latex	Saponin-free sensitization latex
Negative control	Normal healthy human serum	-	-
	Whole blood (anticoagulant-free)	-	?
	Whole blood (heparinized)	-	?
	Whole blood (ACD blood)	-	?
Positive control	RA Serum	+	+
	Whole blood (anticoagulant-free)	+	?
	Whole blood (heparinized)	+	?
	Whole blood (ACD blood)	+	?

Based on the results shown in Table 1, the saponin-supplemented sensitization latex test solution enabled a definitive judgement even when using as a sample a whole blood, revealing an excellent specificity.

IV. Typical effects of the invention

According to the invention, a method for measuring the blood level of an antigen or antibody by which the antigen or antibody level can directly be measured using a while blood.

Since the inventive method employs a hemolytic agent to lyze red blood cells, the aggregation can readily be judged to be present or absent even when using a whole blood as a sample. Accordingly, it eliminates the need of preparation of a serum sample which is essential for an aggregation test in a conventional method, whereby simplifying the measurement.

The invention also provides a reagent employed preferably in the measurement described above. Since the inventive reagent contains a hemolytic agent and an antigen- or antibody sensitization carrier, it lyzes red blood cells only by mixing it with a whole blood, whereby allowing the aggregation test to be conducted easily.

PATENT ABSTRACTS OF JAPAN

(11)Publication number : 60-047962
(43)Date of publication of application : 15.03.1985

(51)Int.Cl. G01N 33/543
A61K 39/44

(21)Application number : 58-154922
(22)Date of filing : 26.08.1983

(71)Applicant : TERUMO CORP
(72)Inventor : ITO YOSHITAKA

(54) METHOD FOR MEASURING AMOUNT OF ANTIGEN OR ANTIBODY IN BLOOD AND TEST SOLUTION USED THEREIN

(57)Abstract:
PURPOSE: To make it possible to directly measure the amount of an antigen or antibody from whole blood by omitting labor for preparing serum, by adding an antigen or antibody sensitized carrier floated solution of a hemolytic agent to a whole blood specimen, and tracking the agglutination reaction thereof.
CONSTITUTION: Saponin or various surfactants are used as a hemolytic agent. The hemolytic agent may be preliminarily added to whole blood prior to agglutination reaction to dissolve a red corpuscle or preliminarily added to an antigen or antibody sensitized carrier floated solution in a concn. of about 0.2W2% to dissolve the red corpuscle at the time of agglutination reaction. As the antigen or antibody sensitized carrier, a latex resin, an inorg. adsorbent or an immobilized red corpusele treated with chemicals can be used. The tracking of agglutination reaction is performed according to a usual method. That is, one drop of whole blood is dripped on a glass slide and one drop of the hemolytic agent or the antigen or antibody sensitized carrier floated solution is added to said hemolytic agent while both of them are well mixed by a wooden rod and spread in a size of 20 × 25mm. The glass slide is held by both hands to be shaken and, thereafter, the presence of absence of agglutination is judged with the naked eye.

LEGAL STATUS

[Date of request for examination]

[Date of sending the examiner's decision of rejection]

[Kind of final disposal of application other than the examiner's decision of rejection or application converted registration]

[Date of final disposal for application]

[Patent number]

[Date of registration]

[Number of appeal against examiner's decision of rejection]

[Date of requesting appeal against examiner's decision of rejection]

[Date of extinction of right]

Papers Filed herewith on: 10/30/03

DOCKET NO.: 0397-0431P ATTY: MSW/KJR 42

APPLICANT(S): Shinya Uchida et al.

APPLN. NO: 09/915,580 FILED: 7/27/01

PAT NO.:

- ☐ New Application with Transmittal Letter
- ☐ Utility ☐ Design ☐ CIP ☐ PCT ☐ Provisional
- ☐ Filing Under 37 CFR 1.53(b) ☐ CONT ☐ DIV
- ☐ Filing Under 37 CFR 1.114(RCE)
- ☐ Specification Consisting of: ______ pages
- ☐ Combined Declaration & Power of Attorney
- ☐ Assignment / Cover Letter
- ☐ Letter to Official Draftsman
- ☐ Drawings ___ Sheets ☐ Formal ☐ Informal ☐ Red-Ink
- ☐ Completion of Filing Requirements, PCT/DO/EO/905 or Formalities Letter and Executed Declaration
- ☐ Priority Document(s) / Cover Letter, No. Doc. ______
- ☒ Amendment: ______
- ☒ Transmit Ltr ☒ Large Entity ☐ Small Entity
- ☐ Response ______
- ☐ Information Discl Stmnt, PTO-1449(s) ______ doc(s)
- ☐ PTO / ISA / 210
- ☐ Notice of Appeal ☐ Appeal Brief
- ☐ Issue Fee Transmittal ☐ Sequence Listing
- ☐ FEES: ______
- ☐ Letter: ______
- ☒ Other: Translation of JPA 60-47162

DOCKET NO. 0397-0431P

O I P E
OCT 3 1 2003
PATENT & TRADEMARK OFFICE

Receipt is hereby acknowledged of the papers filed as indicated in connection with the above identified case.
COMMISSIONER OF PATENTS AND TRADEMARKS

Due Date: 11/4/03

Handcarry: ______